CLAIMS:

1. A purified secreted *Chlamydia* polypeptide which is identified by its expression by a Gram-negative bacterial strain and secretion by the type III secretion pathway of said bacterial strain.

2. A purified polypeptide according to Claim 1 wherein said polypeptide is selected by a method for identifying polypeptides secreted by *Chlamydia* comprising (a) providing a recombinant expression vector containing at least the DNA coding for the polypeptide of interest; (b) transforming a Gram-negative strain containing a type III secretion pathway with said recombinant vector; (c) expressing this vector in the Gram-negative strain transformed in (b); and (d) detecting the secretion of said DNA expression product; wherein the secretion of said expression product indicates that it corresponds to a secreted *Chlamydia* polypeptide.

3. A purified polypeptide according to Claim 1 wherein said polypeptide is selected by a method for identifying polypeptides secreted by *Chlamydia* comprising (a) providing a recombinant expression vector comprising at least the DNA coding for the polypeptide of interest fused to a reporter gene; (b) transforming a Gram-negative strain containing a type III secretion pathway with said recombinant vector; (c) expressing this vector in the Gram-negative strain transformed in (b); and (d) detecting the secretion of said reporter gene expression product; wherein the secretion of said expression product indicates that the fused DNA contains at least a polynucleotide corresponding to a secreted *Chlamydia* polypeptide.

4. A purified secreted *Chlamydia* polypeptide according to Claims 1, 2 or 3 wherein said Gram-negative strain containing a type III secretion pathway is a *Shigella* strain.

5. A purified polypeptide according to Claim 1 wherein said polypeptide belongs to the Inc family.

6. A purified polypeptide according to Claim 5 wherein said polypeptide is selected from the group consisting of IncA, IncB, IncC, CPn0026, CPn0308 and CPn0585.

7. A method for identifying a secreted *Chlamydia* polypeptide wherein said method comprises (a) providing a recombinant expression vector containing at least DNA coding for the polypeptide of interest; (b) transforming a Gram-negative strain containing a type III secretion pathway with said recombinant vector; (c) expressing said vector in said Gram-negative transformed strain; and (d) detecting the secretion of said DNA expression product; wherein the secretion of said expression product indicates that it corresponds to a secreted *Chlamydia* polypeptide.

8. A method for identifying a secreted *Chlamydia* polypeptide wherein said method comprises (a) providing a recombinant expression vector containing at least DNA coding for the polypeptide of interest fused to a reporter gene; (b) transforming a Gram-negative strain containing a type III secretion pathway with said recombinant vector; (c) expressing this vector in said transformed Gram-negative strain; and (d) detecting the secretion of said reporter gene expression product; wherein the secretion of said expression product indicates that the fused DNA contains at least a polynucleotide corresponding to a secreted *Chlamydia* polypeptide.

9. A method according to Claims 7 or 8 wherein said Gram-negative strain containing a type III secretion pathway is a *Shigella* strain.

10. A method according to Claims 7 or 8 wherein said expression product is secreted by a type III secretion pathway.

11. A method for screening an active molecule inhibiting the secretion of a secreted *Chlamydia* polypeptide wherein said method comprises (a) providing a recombinant expression vector containing at least DNA coding for the polypeptide the secretion of which is to be inhibited; (b) transforming a Gram-negative strain containing a type III secretion pathway with said recombinant vector; (c) expressing said DNA of said vector in said transformed Gram-negative strain in the presence of the tested molecule; (d) expressing said DNA of said vector in said transformed Gram-negative strain in the absence of the tested molecule; and (e) comparing secretion of the DNA expression product of step (c) and step (d); wherein a decrease of said secretion is indicative of the ability of said tested molecule to inhibit secretion of said secreted *Chlamydia* polypeptide.

12. A method for screening a molecule which inhibits secretion of a secreted *Chlamydia* polypeptide wherein said method comprises (a) providing a recombinant expression vector containing at least DNA coding for the polypeptide the secretion of which is to be inhibited fused to a reporter gene; (b) transforming a Gram-negative strain containing a type III secretion pathway with said recombinant vector; (c) expressing said vector in said transformed Gram-negative strain in the presence of the tested molecule; (d) expressing said vector in said transformed Gram-negative strain in the absence of the tested molecule; and (e) comparing secretion of the expression product of said reporter gene in step (c) and step (d); wherein a decrease of said secretion is indicative of the ability of said tested molecule to inhibit secretion of said secreted *Chlamydia* polypeptide.

13. A method according to Claims 11 or 12 wherein said Gram-negative strain containing a type III secretion pathway is a *Shigella* strain.

14. A method according to Claims 11 or 12, wherein the tested molecule inhibits type III pathway secretion.

15. An immunogenic composition comprising at least a polypeptide according to Claim 1 or an immunogenic fragment thereof.

16. A vaccinating composition against *Chlamydia* infection wherein said composition comprises at least one polypeptide according to Claim 1 or an immunogenic fragment thereof along with a pharmaceutically acceptable carrier.

17. A vaccinating composition according to Claim 16, wherein said infection contributes to atherosclerosis.

18. The vaccinating composition according to Claim 16, wherein said infection is a sexually transmitted disease.

19. A therapeutic composition active against *Chlamydia* infection, wherein said therapeutic composition comprises at least an active molecule identified by the method according to Claims 11 or 12.

20. An antibody against *Chlamydia* wherein said antibody is directed against the polypeptide according to Claim 1 or an antigenic fragment thereof.

21. A method for diagnosing a *Chlamydia* infection in a patient wherein said method comprises (a) providing a polypeptide according to Claim 1, or an immunogenic fragment thereof, optionally labeled; (b) bringing said polypeptide or immunogenic fragment thereof into contact with a serum sample of said patient; and (c) detecting complexes formed between said polypeptide or immunogenic fragment thereof and antibodies contained in the serum sample; wherein said complexes are indicative of a *Chlamydia* infection in said patient.

22. A method for diagnosing a *Chlamydia* infection in a patient wherein said method comprises: (a) providing a patient sample of a tissue suspected to be infected by

Chlamydia; (b) bringing said sample into contact with an antibody according to Claim 20; and (c) detecting antigen-antibody complexion; wherein said complexion is indicative of a *Chlamydia* infection in said patient.

23. A plasmid for expression of secreted *Chlamydia* polypeptide wherein said plasmid contains at least DNA coding for a polypeptide according to Claim 1.

24. A plasmid according to Claim 22 wherein said DNA is further fused to a reporter gene.

25. The plasmid of Claim 24, wherein a vector deposited at C.N.C.M. on December 13, 2000 with accession No. I-2593 is used for the construction of said plasmid.

26. A recombinant Gram-negative strain wherein said strain is transformed by a plasmid according to Claim 23.

27. A recombinant Gram-negative strain according to Claim 26 wherein said strain is a *Shigella* strain.

28. A recombinant Gram-negative strain according to Claim 26 wherein said strain contains the DNA coding for an IncA polypeptide, said strain being deposited at C.N.C.M. with accession No. I-2592 on December 13, 2000.

29. A method of preventing or treating a *Chlamydia* infection in a mammal, preferably a human, which comprises administering an effective amount of a purified secreted polypeptide of *Chlamydia* which is identified by its secretion in a Gram-negative strain containing a type III secretion pathway to a mammal in need thereof.

ABSTRACT

Chlamydia spp. are strictly intracellular pathogens that grow inside a vacuole, called an inclusion. They possess genes encoding proteins homologous to components of type III secretion machineries which, in other bacterial pathogens, are involved in delivery of bacterial proteins within or through the membrane of eukaryotic host cells. Inc proteins are chlamydial proteins that are associated with the membrane of the inclusion and are characterized by the presence of a large hydrophobic domain in their amino acid sequence. To investigate whether some *Chlamydia* proteins, especially Inc proteins and other proteins exhibiting a similar hydropathic profile, might be secreted, the inventors used an heterologous secretion system, namely a type III system. Chimeras were constructed by fusing the N-terminal part of these proteins with a reporter, the Cya protein of *Bordetella pertussis*, and expressed in various strains of *Shigella flexneri*. The inventors demonstrate that these hybrid proteins are secreted by the type III secretion system of *S. flexneri*. Moreover, the inventors show that three other proteins from *C. pneumoniae*, all of which have in common the presence of a large hydrophobic domain, are also secreted by *S. flexneri* type III secretion machinery.

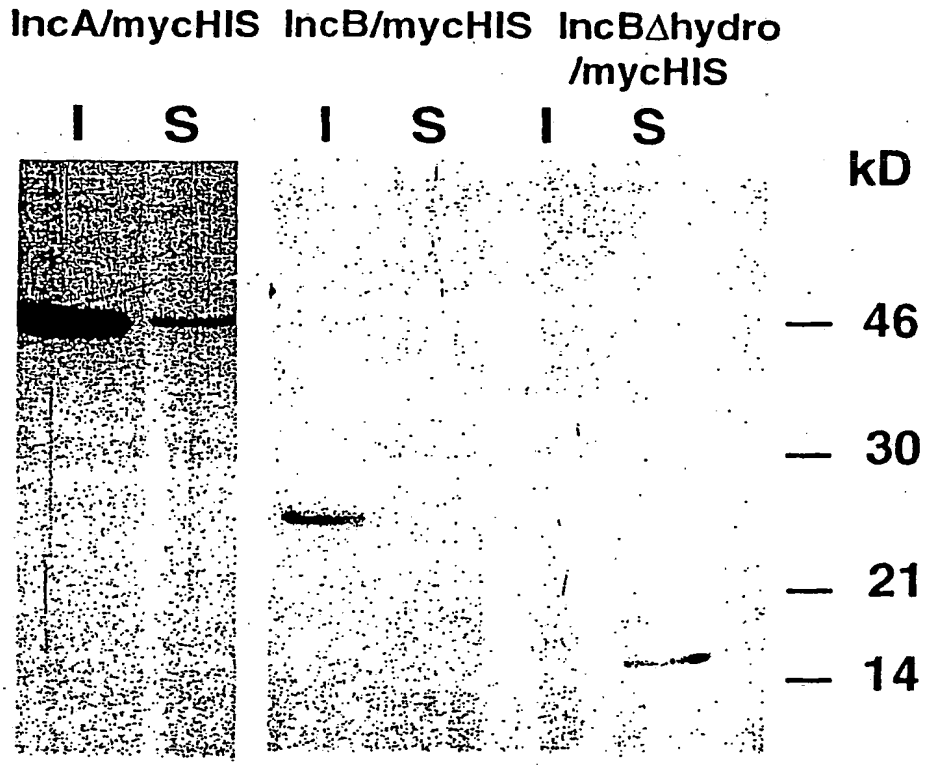
IncA/mycHIS
IncB/mycHIS
IncBΔhydro /mycHIS
I S I S I S
kD
46
30
21
14

FIGURE 1

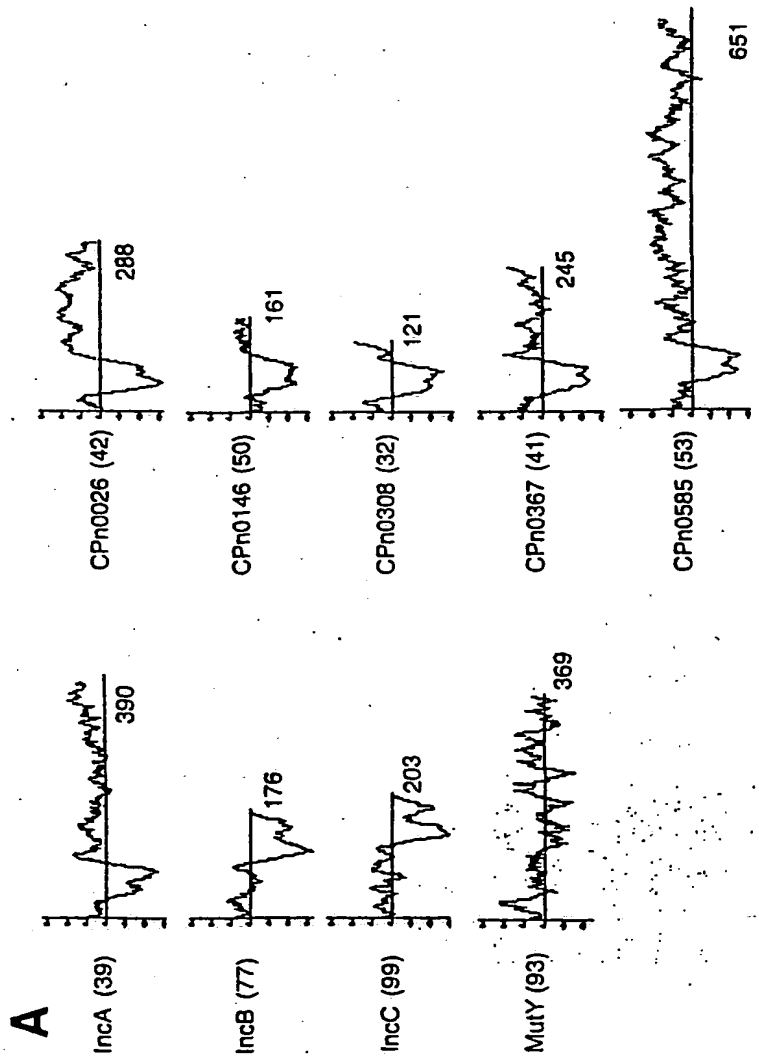
A
IncA (39)
390
IncB (77)
176
IncC (99)
203
MutY (93)
369
CPn0026 (42)
288
CPn0146 (50)
161
CPn0308 (32)
121
CPn0367 (41)
245
CPn0585 (53)
651

FIGURE 2A

FIGURE 2C

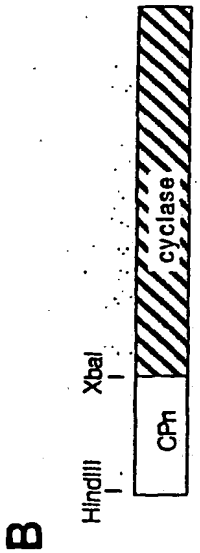

FIGURE 2B

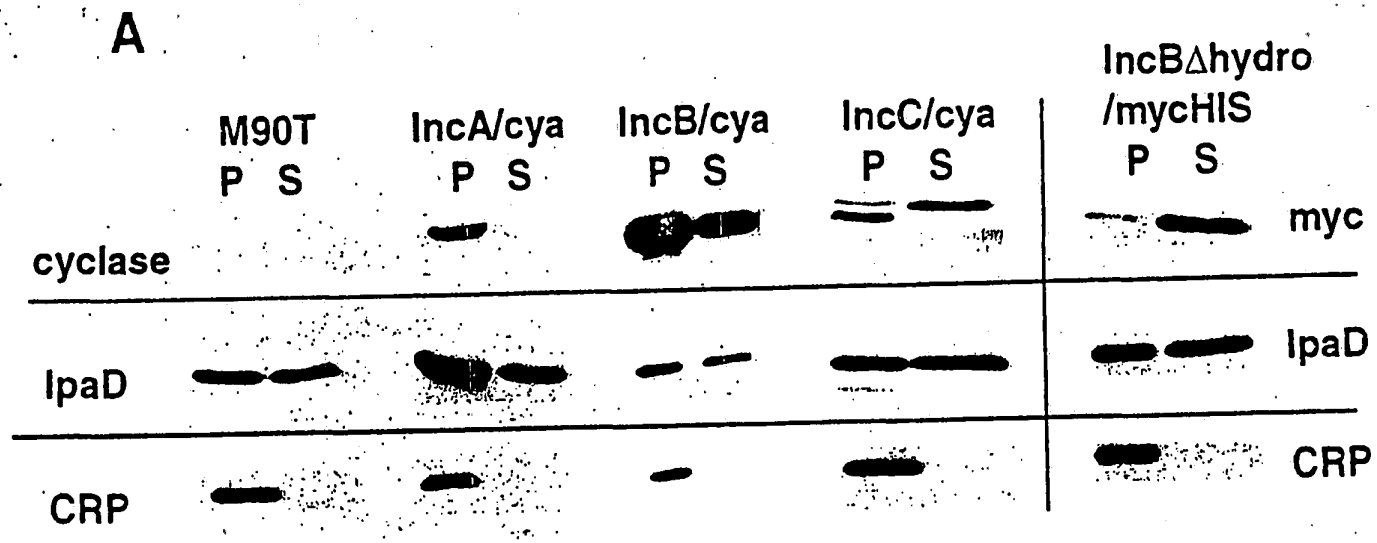
A
M90T
P S
IncA/cya
P S
IncB/cya
P S
IncC/cya
P S
IncBΔhydro
/mycHIS
P S
cyclase
myc
IpaD
IpaD
CRP
CRP

FIGURE 3A

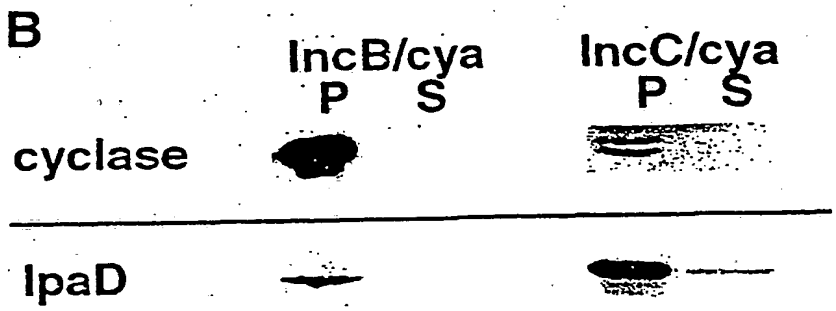

FIGURE 3B

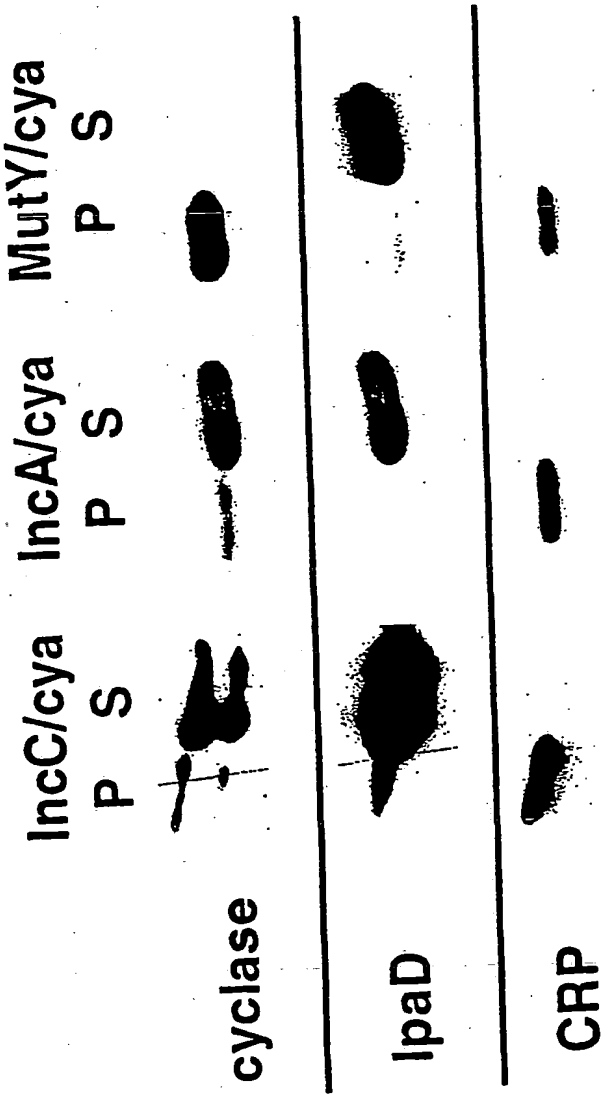

FIGURE 4

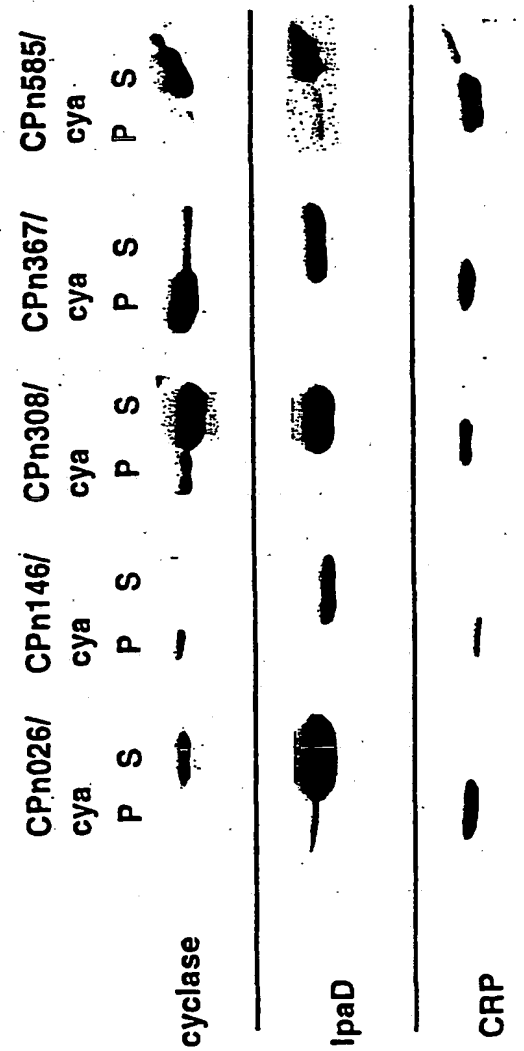
CPn026/ cya
P S
CPn146/ cya
P S
CPn308/ cya
P S
CPn367/ cya
P S
CPn585/ cya
P S
cyclase
IpaD
CRP

FIGURE 5